DOI: 10.2478/s11686-012-0010-0

Acta Parasitologica, 2012, 57(1), 7–12; ISSN 1230-2821

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Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in dogs from Korea

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Abstract

Toxoplasma gondii and *Neospora caninum* are closely related protozoan parasites, they share many common hosts, and can cause neurological diseases in dogs. Dogs can have close contacts with humans and livestock and therefore they can act as reservoirs of these parasites. The aim of this study was to survey the seroprevalence of antibodies against *T. gondii* and *N. caninum* and their co-infection rate in dogs in Korea. In total, sera from 553 domestic dogs were collected from different breeds, sexes, and ages of dogs from nine provinces across the country of Korea during 2006 and 2007. The presence of antibodies against *T. gondii* and *N. caninum* was analyzed using the latex agglutination test (LAT) with a cut-off value of 1:32, and the indirect fluorescent antibody test (IFAT) using a serum titer of 1:100. In the total dog population, 71 (12.8%) dogs were positive for anti-*T. gondii* antibodies and only 20 (3.6%) were positive for anti-*N. caninum* antibodies. Relatively higher seropositive frequencies of antibodies against *T. gondii* (20.1%) and *N. caninum* (4.9%) were detected in the dog population from the Gyeonggi. A higher proportion of animals seropositive for anti-*T. gondii* antibodies was found in stray dog populations as compared to household dog populations: 18.5% (59/319) vs 5.1% (12/234), respectively. The Chi-square tests revealed significant differences in the seropositive frequencies of antibodies against *T. gondii* between stray and household dogs in the total population ($p<0.0001$), and in dogs from the Gyeonggi ($p<0.01$). No significant differences were observed for the presence of antibodies against *T. gondii* or *N. caninum* when compared across the sex or age ($p>0.05$). The first serological survey on antibodies against both *T. gondii* and *N. caninum* parasites across the entire country showed that co-infection was not common in these canine populations with a seropositive level of 0.72%. The significantly higher positive frequency of *T. gondii* antibodies in stray dogs in both, Gyeonggi and in the total dog populations suggests that further investigation on the seroprevalence of parasites should focus on stray dogs.

Keywords

Dog, *Toxoplasma gondii*, *Neospora caninum*, seroprevalence, Korea

Toxoplasma gondii is one of the most common parasites in animals in which felids are the only primary hosts and warm-blooded animals are the intermediate hosts (Frenkel *et al*. 1970). Infection with *T. gondii*, called toxoplasmosis, can be very serious in humans, especially in pregnant woman and humans with immunodeficiency. *Neospora caninum* is a coccidian parasite, and its oocysts have been found in feces of domestic dogs (*Canis familiaris*) and coyotes (*Canis latrans*). Therefore, dogs are definitive hosts of *N. caninum* and play a vital role in the cycle of transmission to other animals (Dubey 1999). It was confirmed that *N. caninum* is an important risk factor of abortion and infertility in dairy cattle worldwide (Dubey 2003). Domestic dogs are in contact with humans, pets and livestock such as cat, cattle, sheep, and are considered a reservoir host for the transmission of both parasites. Therefore, the presence of antibodies against *T. gondii* or *N. caninum* and the coexistence of both parasites in dogs have been investigated in several studies. The prevalence of anti-*N. caninum* and anti-*T. gondii* antibodies in dogs varies greatly among different geographical regions. Recent surveys showed that the number of *T. gondii* seropositive dogs was as low as 21% in China (Zhang *et al*. 2010) and as high as 69.8% in Brazil (Valadas *et al*. 2010). For *N. caninum*, the level ranged from 1.9% in the West Indies (Dubey *et al*. 2008), to an intermediate percentage of 8.4% in Northeast Brazil (Azevedo *et al*. 2005), and to the highest value of 32% in Iran (Hosseininejad *et al*. 2010). However, the reported comparison of prevalence data depends on numerous factors, such as the number, type and origin of dogs

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tested, and the sensitivity and specificity of the serological tests applied (Dubey *et al*. 1985, Silva *et al*. 1997). In Korea, the seroprevalence of *T*. *gondii* and/or *N*. *caninum* was previously studied in cats (Kim *et al*. 2008, Lee *et al*. 2010), cattle (Bae *et al*. 2000, Kim *et al*. 2002), dogs (Kim *et al*. 2003, Lee *et al*. 2008) and humans (Han *et al*. 2008). The aim of this study was to survey the seropositive frequency of antibodies against *T*. *gondii* and/or *N*. *caninum* in stray and household dogs from Korea. Moreover, the impacts of other epidemiological factors such as sex, age and life-style (i.e. stray vs household dogs) on the seropositive rate of antibodies to both parasites were also considered.

Samples were collected from 553 domestic dogs of different breeds, sexes and ages from all nine provinces of Korea

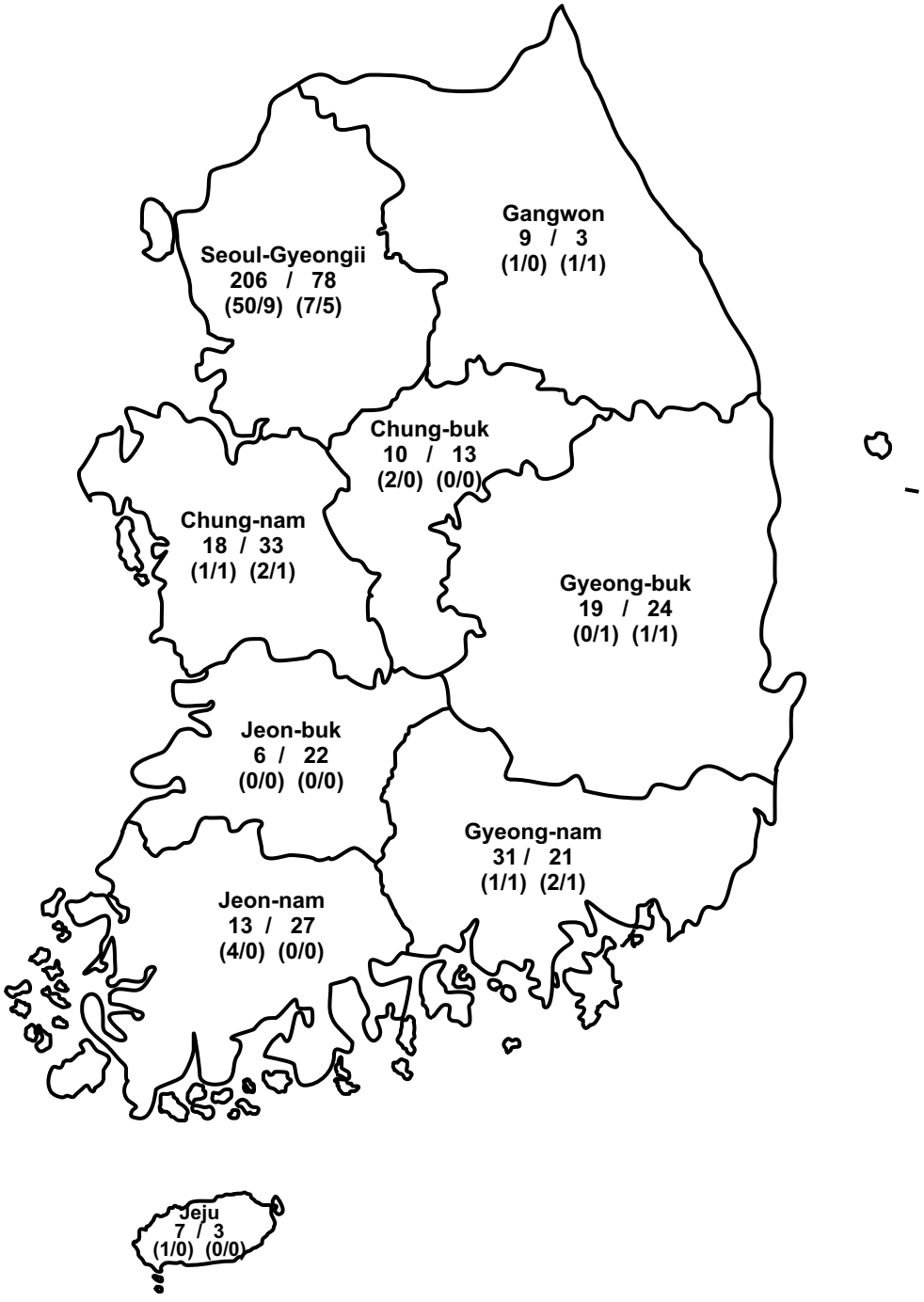

Fig. 1. Korean map showing locations and numbers of sample collection (number of stray/household dogs collected). Numbers in parentheses correspond to the number of dogs seropositive for anti-*T gondii* and anti-*N. caninum* antibodies, respectively, in each dog group

during January 2006 and October 2007. Among them, 319 were stray dogs collected by local veterinarians and 234 were household dogs from clinical hospitals. Blood samples were taken from the cephalic vein and placed in vacuum tubes without anti-coagulant, and sera were then separated and stored at –20°C until use. Epidemiological data (breed/sex/age/ lifestyle) of each dog were recorded by veterinarians.

For detection of *T. gondii* antibodies, the in-house latex agglutination test (LAT) was performed. *T. gondii* tachyzoites were cultivated in Vero cells using RPMI 1640 medium at 37°C, 5% CO_2 for 3 days. The culture medium was filtered with 3-µm Whatman paper, and then centrifuged to harvest the cells. After three washes with PBS, the pellet was sonicated for 3 min in two-second intervals, and then the supernatant was used for the LAT. In each well, 25 µl latex beads (Polybead®Polystyrene Dyed Red 1.0 Micron Microspheres – Polysciences Inc.) was coated with *T. gondii* lysate (300 µg/ml total protein concentration) at a ratio of 1:1 (volume/volume), and then blocked with 10× BSA at 37°C for 3 hours. A positive/negative serum for *T. gondii* produced in rabbit (NVRQS, Korea) was used as a control. The different sera dilutions were tested and a serum titer of 1:32 was used as a cut-off value. Dog sera at different dilutions were checked using the commercial latex kit of Toxotest-MT (Eiken Chemical Co. Ltd., Japan). The commercial kit cut-off value of 32× showed the comparable results.

An indirect fluorescent antibody test (IFAT) was performed to detect anti-*N. caninum* antibodies in dog sera. Slides were coupled with *N. caninum* tachyzoites maintained in Vero cells cultured in our lab. Dog sera were screened at a titer of 1:50 cut-off value. Purified anti-dog immunoglobubin (whole IgG) produced in goat and labeled with fluorescein isothiocyanate – (FITC; KPL, USA) was used as the conjugate. On each slide, sera from canine positive to *N. caninum* and from canine negative to *N. caninum* (VMRD Inc., USA) were included as a control.

Data were analyzed according to life-style (stray/household dogs), sex (male/female), and age (<1 year/older). To determine the differences in prevalence among groups, the Chi-square test was applied by using GraphPad InStat software, version 3.10 (Motulsky 2009); p values less than 0.05 were considered statistically significant.

In this study, 553 dogs were tested for the presence of antibodies against *T. gondii* and *N. caninum*. The location, the number of tested dogs belonging to the stray and household groups and the distribution of seropositive dogs are presented in Figure 1. As shown in Figure 1, the number of dogs positive for both parasites was different in each region. The seroprevalence of anti-*T. gondii* and anti-*N. caninum* antibodies are shown in Table I. The overall positive value was 12.8% (71/553) for anti-*T. gondii* antibodies and 3.6% (20/553) for anti-*N. caninum* antibodies. In the total dog population, a similar number of animals positive for antibodies against *T. gondii* in females (12.7%) and males (13.0%) was observed. The values in age categories were also not significantly different, with 10.2% in dogs less than one year old and 14.5% in older dogs ($p>0.05$). For anti-*N. caninum* antibodies, comparison of the positive frequency of female (3.3%) vs male dogs (3.9%) and dogs younger than one year (3.9%) vs dogs older than one year (3.5%) showed no significant difference.

Table II shows the frequencies of antibodies against *T. gondii* and *N. caninum* in sera of stray and household dogs categorized by sex and age. Although both groups of dogs were positive for *T. gondii* antibodies, the seroprevalence in

Table I. Prevalence of anti-*T. gondii* and anti-*N. caninum* antibodies in dogs

Location	Epidemiological factors	Total number	*T. gondii*		*N. caninum*	
			Positive	%	Positive	%
Total dogs	**Sex**					
	Female	245	31	12.7 ns	8	3.3 ns
	Male	308	40	13.0 ns	12	3.9 ns
	Age					
	<1 year	207	21	10.2 ns	8	3.9 ns
	Older	346	50	14.5 ns	12	3.5 ns
	Total	553	71	12.8	20	3.6
Gyeonggi dogs	**Sex**					
	Female	128	28	21.8 ns	8	6.3 ns
	Male	156	29	18.6 ns	5	3.2 ns
	Age					
	<1 year	81	16	19.8 ns	5	6.2 ns
	Older	203	41	20.2 ns	8	3.9 ns
	Total	284	57	20.1	13	4.6

(%) of positive dogs; ns – no significant difference (Chi-square test).

Table II. Prevalence of anti-*T. gondii* and anti-*N. caninum* antibodies in stray and household dogs categorized by sex/age

Factors	Number of tested dogs	Stray dogs				Household dogs			
		T. gondii		*N. caninum*		*T. gondii*		*N. caninum*	
		Positive	%	Positive	%	Positive	%	Positive	%
Sex									
Female	137/108#	26	18.9 ns	5	3.6 ns	5	4.6 ns	3	2.8 ns
Male	182/125#	33	18.1 ns	6	3.3 ns	7	5.6 ns	6	4.8 ns
Age									
<1 year	103/100#	19	18.5 ns	5	4.9 ns	2	2.0 ns	3	3.0 ns
Older	216/134#	40	18.5 ns	6	2.8 ns	10	7.5 ns	6	4.5 ns
Total dogs	(319/234) 553	59	18.5**	11	3.5 ns	12	5.1**	9	3.9 ns
Sex									
Female	96/32	25	26.4 ns	6	6.3 ns	3	9.4 ns	2	6.3 ns
Male	110/46	25	22.7 ns	3	2.7 ns	4	8.7 ns	2	4.3 ns
Age									
<1 year	72/9	16	22.2 ns	5	6.9 ns	0	0†	0	0†
Older	134/69	34	25.4 ns	4	3.0 ns	7	10.1†	4	5.8†
Gyeonggi dogs	(206/78) 284#	50	24.3*	9	4.4 ns	7	9.0*	4	5.1 ns

#The number of stray/household dogs; (%) of positive dogs; **extremely significant difference ($p<0.0001$); *significant difference ($p<0.01$); ns – no significant difference; †not defined.

stray dogs (18.5%) was significantly higher ($p<0.0001$) than that in household dogs (5.1%). In the anti-*N. caninum* antibody tests, similar positive frequencies were found in stray dogs (3.5%) and in household dogs (3.9%). The frequency of antibodies against *T. gondii* and *N. caninum* when comparing sex and age in both dog populations was not significantly different ($p>0.05$), e.g., 18.9% vs 18.1% for female and male stray dogs, respectively, and 4.6% vs 5.6% household dogs, respectively (Table II).

In the Gyeonggi, where approximately 50% of total Korean human population lives, higher seropositive frequencies of antibodies against *T. gondii* (20.1%, 57/283) and *N. caninum* (4.9%, 14/283) were observed in comparison to that of the total dog population. Dogs positive for anti-*T. gondii* antibodies were found at frequency of 50 out of 206 stray dogs and 7 out of 77 household dogs. These data indicated the extremely significant difference between stray dogs (24.3%) and household dogs (9.1%) positive for anti-*T. gondii* antibodies ($p = 0.0076$). The positive frequency of anti-*N. caninum* antibodies in household dogs (6.5%) was higher than that in stray dogs (4.4%), but the Chi-square test did not show statistically significant difference ($p>0.05$). Antibodies for both parasites were found concomitantly in four dogs (0.72%); all these dogs came from the Gyeonggi. Three of them were stray dogs and one was a household dog. The co-infected stray dogs were older than two years and of the Poodle breed.

The results of this study indicate that dogs of two life styles (i.e. stray and household) in Korea were seropositive for anti-*T. gondii* and anti-*N. caninum* antibodies. Gyeonggi is the most human population-dense province; therefore, the seroprevalence of *T. gondii* and *N. caninum* antibodies in dogs from this region was analyzed separately. The overall seroprevalence of anti-*T. gondii* antibodies found in our investigation was 12.8%, which is much lower than what was recently reported in many other countries (Azevedo *et al*. 2005, Figueredo *et al.* 2008, Valadas *et al*. 2010). The comparable positive frequencies of *N. caninum* antibodies were observed in dogs from Gyeonggi (4.6%) and the total population (3.6%), which were as low as those reported by Wanha *et al*. (2005) in which *N. caninum* antibodies were found in 3.6% of dog from Austria (Wanha *et al*. 2005). The low seroprevalence of antibodies against *N. caninum* versus the high prevalence of that of *T. gondii* found in this study supports the fact that these antigens are distinct. This finding also indicates that the *T. gondii* and *N. caninum* serological tests are specific (Dubey *et al*. 2007).

Epidemiological factors such as the life-style (stray/household dogs), sex (female/male), and age (<1 year/older) were evaluated in this study. Regarding the life-style, the higher seroprevalence of *T. gondii* antibodies in stray dogs when compared to household dogs found in Gyeonggi (24.3% vs 9.0%) and in total dogs (18.5% vs 5.1%) seems to be due to the fact that stray dogs are in closer contact with contamination sources and other intermediate hosts of *T. gondii*. Our results are in agreement with the relatively high seroprevalence (16.1%) of antibodies against *T. gondii* observed in stray cats from the Gyeonggi (Kim *et al*. 2008). Although *T. gondii* is not considered a primary pathogen in dogs, the relatively high seroprevalence detected in dogs from several surveys is probably because stray and household dogs are more closely in contact with stray and household cats, respectively, which were confirmed as the definitive host for *T. gondii*. In contrast

to previous studies, the lower seropositive frequencies of *N. caninum* in stray dogs and higher values in household dogs from Gyeonggi and the total population were observed, e.g., antibodies against *N. caninum* were found at a higher in dogs from dairy farms (21.6%) than in urban dogs (8.3%) from Korea (Kim *et al*. 2003).

Regarding the age and sex of dogs, this study did not show any differences in seropositive frequencies for antibodies against *T. gondii* or *N. caninum* ($p>0.05$), which is in agreement with many previous studies (Azevedo *et al*. 2005, Gennari *et al*. 2006, Dubey *et al*. 2007, Zhang *et al*. 2010). From the epidemiological factors analyzed, the role of lifestyle (stray/household/rural/urban/farm) was confirmed to be the most important factor affecting the seropositive frequency of antibodies against *T. gondii* and *N. caninum* in dogs in many studies.

The seropositivity of antibodies against both parasites found in this study (0.72%) was relatively low when compared with seroprevalence data described previously, such as 5.7% (Mineo *et al*. 2004); 4.9% (Azevedo *et al*. 2005), 20.8% (Romanelli *et al*. 2007), and 15.7% (Figueredo *et al*. 2008, Yildiz *et al*. 2009), showing that the co-presence of antibodies against *T. gondii* and *N. caninum* is not common in dogs from Korea. This is the first serological survey on both *T. gondii* and *N. caninum* across the entire country. The relatively higher frequency of seropositivity for antibodies against *T. gondii* in dogs found in this study suggests that further investigation on the seroprevalence of those parasites should be encouraged and be focused on stray dogs.

Acknowledgements. This study was financed by a grant from the National Veterinary Research and Quarantine Service (NVRQS), Korean Ministry of Agriculture and Forestry, Korea.

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(Accepted January 17, 2012)